IMPERIAL BUREAU OF PLANT GENETICS: HERBAGE PLANTS

BULLETIN No. 7.

FURTHER CONTRIBUTIONS ON THE TECHNIQUE EMPLOYED IN THE BREEDING OF HERBAGE AND FORAGE PLANTS.

Price 2/-

AGRICULTURAL BUILDINGS,
ABERYSTWYTH,
WALES.

MAY, 1932.

Cambrian News, Aberystwyth.

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HERBAGE ABSTRACTS.—A quarterly review of literature on herbage plants, forage crops and grassland research. [1s. 6d. per copy or 5s. per annum]. [Vol 1. No. 1, out of print].

BULLETIN SERIES (Irregular).

Bull. No. 1. Miscellaneous Information relating to Breeding of Herbage Plants. March 1930. [Out of print].
Bull. No. 2. Miscellaneous Information relating to Herbage Plants. September 1930. [Out of print].
Bull. No. 3. The Breeding of Herbage Plants: Technique adopted at the Welsh Plant Breeding Station. June 1931. (3s).
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CONTENTS.

PAGE.

I. Methods employed in the breeding of biennial sweet clover (*Melilotus*) and brief notes on the breeding of lucerne (*Medicago sativa*), brome grass (*Bromus inermis*) and slender wheat grass (*Agropyron tenerum*), by L. E. Kirk 5

II. The breeding of *Cicer* and *Cajanus*, by F. J. F. Shaw 14

III. The improvement of *Phalaris* species by selective breeding, by H. C. Trumble .. 17

IV. Breeding technique for *Pennisetum typhoideum*, by W. Burns and D. B. Barve .. 21

V. Methods employed in pure line breeding of insect-pollinated plants : Red Clover, by I. S. Travin 22

VI. Brief notes on seed setting in lucerne, by R. E. Dwyer 32

INTRODUCTORY NOTE.

The present Bulletin contains contributions from forage crop workers in various parts of the world on certain questions of technique which have arisen in the breeding of numerous different crops under a wide range of climatic conditions. These notes had of necessity to be brief; it is not possible at this stage to prepare a detailed symposium on the subject, but it is hoped that the following articles will help towards a fruitful interchange of opinions and ideas. Further, not all the crops discussed are primarily of forage value, but in these cases the results are applicable to nearly related forage crops, for which reason they were included.

Among points of special interest which might be referred to are the use of artificial light in work on *Melilotus* (Kirk), the low rate of natural cross-fertilization in *Cajanus indicus* (Shaw), the valuable report on the work on *Phalaris*, including the methods adopted in distinguishing the seed of *P. minor* (Trumble) and the notes on Soviet red clover breeding (Travin). Although red clover was discussed very fully in our Bulletin No. 3, it was thought that these additional notes would be of value, especially in connection with the difficulties encountered owing to the severity of winter conditions.

R.O.W.

I.—METHODS EMPLOYED IN THE BREEDING OF BIENNIAL SWEET CLOVER (*MELILOTUS*) AND BRIEF NOTES ON THE BREEDING OF LUCERNE (*MEDICAGO SATIVA*), BROME GRASS (*BROMUS INERMIS*) AND SLENDER WHEAT GRASS (*AGROPYRON TENERUM*).

BY

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In the contribution which I shall make to these discussions on the methods and technique of breeding herbage plants, I will deal chiefly with sweet clover (*Melilotus*), since this crop has attained much greater importance in America than elsewhere and because it is not likely to receive attention in this publication by any other worker. The other species with which I have worked quite extensively are lucerne (*Medicago sativa*), brome grass (*Bromus inermis*), and slender wheat grass (*Agropyron tenerum*). The methods employed with these plants will be referred to very briefly.

Sweet Clover (Melilotus).

Plant Material.

There are only two species of *Melilotus* that are used to any extent in America as cultivated crops, namely, the white and yellow flowered biennial species. These are commonly designated *M. alba* and *M. officinalis* respectively, although within each are to be found several very distinctive strains. Some of these have been referred to as different species, and are probably deserving of specific rank. Until more is known, however, with respect to the taxonomy of the genus, there seems no good reason for the plant breeder to distinguish more than two species.

The fact remains that the wide variations in plant type and habit of growth which exist in both white flowered and yellow flowered sweet clover provide an excellent opportunity for the selection of material for different agricultural zones and different purposes. The plant breeder who is making a beginning with this crop would do well, therefore, to start with a fairly wide range of varieties or strains, rather than confine his attention exclusively to named varieties or commercial material.

Objectives.

For the most part sweet clover is not attractive as a hay crop because of the excessively coarse and stemmy character of the herbage. This is the case more especially under favourable moisture conditions, since the plants attain a very large size and rapidly become too stemmy to make hay; also if they are cut at an early stage of growth the herbage is difficult to cure. For this reason, ordinary sweet clover is most valuable as a pasture crop, although it is prized for hay also in areas where the limited precipitation precludes too coarse a growth. One of the objectives, therefore, in sweet clover improvement is to secure types which have finer stems and a greater percentage of foliage.

Some varieties of sweet clover meet the requirements for hay much better than others, notably the "Arctic" variety in Western Canada and the variety "Grundy County" in West Central United States. These two belong to what may be termed the semi-dwarf early maturing group of *M. alba* as opposed to the taller and coarser late maturing types. Varieties of *M. officinalis* may be

grouped also in a general way into two broad classes on a similar basis, although it is possible to recognise a larger number of distinctive types in the yellow-flowered species. But generally speaking all of them are really too coarse for good quality hay, so that any advance toward a well adapted, more dwarf and finer stemmed leafy strain would be in the direction of improvement. The same statements would not apply equally well with respect to their value for pasture.

It has been possible to make considerable progress toward a more desirable hay type by taking advantage of certain mutant forms which occurred in a field of the Arctic variety on the experimental plots. By inbreeding and selection, two strains have been isolated, increased and distributed. These varieties, known as Alpha No. 1 and No. 3, have been described together with the history of their development (**4**). They are characterized chiefly by having numerous short, fine, leafy stems instead of a few coarse, tall and sparsely leaved stems. These are now being tested in a large way on hundreds of farms. Whether or not they satisfy agricultural requirements in their present form, their salient features can be transmitted easily by crossing and thus combined with other strains that are specially adapted to local conditions.

Another objective in breeding sweet clover is to secure strains which are resistant to disease. Both species are vulnerable to a number of pathogens. One of these is very destructive in parts of Western Canada. This phase of the work promises to be of first importance. As an example we have strains which are highly resistant and others highly susceptible to stem canker (*Ascochyta caulicola*).

The bitter flavour of the herbage is another objectionable feature which the plant breeders should be able to improve. We have made some progress along this line.

Although sweet clover is adapted to an exceptionally wide range of soil types and climatic conditions, there is ample evidence that certain types are much better suited to certain areas than to others. Some varieties which are most useful in the western sections of the Great Central Plains do not thrive in the Atlantic coast states, and other varieties which thrive best in the vicinity of Washington, D.C., do not make a healthy growth in Western Canada. Some of them are lacking in winter hardiness. These are extreme examples of local adaptation which serve to emphasize the fact that there is much to be accomplished with this crop by plant breeding methods.

POLLINATION STUDIES.

Before dealing specifically with the standard precedure which has been adopted in the breeding of sweet clover, it may be as well to outline very briefly what we have learned about the mode of reproduction and behaviour in crossing of the two species in question, as this has been largely responsible for the methods of breeding which we have employed.

With the first material which was used in pollination studies, it was found (**1**) that plants of the white-flowered species, for the most part, would set seed freely when protected from the visitation of insects, whereas those of the yellow-flowered species would not set seed without artificial manipulation, and then usually very sparingly. While this may stand as a broad generalization, we now know that non-self-pollinating white-blossom plants are fairly numerous and at least one type of yellow-flowered sweet clover is largely self-pollinating. What is still more important to the plant breeder, it would appear that strains may be obtained which are self-fertilized before insects normally gain access to the flowers (**5, 6**). This mode of reproduction, when combined with other desirable qualities, provides an objective which is worth striving for and suggests the possibility of securing varieties which would be self-fertilized and reasonably immune from contamination with foreign pollen. The advantage is obvious, especially in view of the fact that loss of vigour through inbreeding appears to be a negligible factor.

Another discovery, both interesting and of considerable practical value, was that the white and yellow-flowered species, when grown in close proximity, did not produce natural hybrids (**2**). This

made it possible to carry on work with both species in the same nursery without danger of natural crossing. Later we found that the two species are easily crossed, and probably cross-fertilize freely in the field, but that such unions result only in tiny, abnormal, non-viable seed (**3**).

PLOT TESTS.

The testing of varieties and strains of sweet clover is conducted in replicated plots which vary in size from 1/40 to 1/100 of an acre. It has been found advisable also to have the same material growing concurrently in rod rows spaced 3 feet apart and in single plant rows, consisting of about 40 plants, spaced 3 feet apart each way. By growing each variety or strain in seeded plots, seeded rows and single plant rows, it is usually possible to get a more comprehensive picture of plant type and growth characteristics than when only one or two methods of culture are used. When enough seed is available all three types of plots are seeded ; when there is only a small amount of seed the rod rows and single plant rows are used. Very often, only single plant rows are possible, especially in the breeding nursery, but when there is enough seed, we like to have the rod rows as well, because growth *en masse* gives a very different conception from that obtained from single plants.

BREEDING.

The method adopted is to isolate desirable strains by inbreeding and selection. The matter of loss in vigour through inbreeding with sweet clover is not a disturbing factor.

When it has been decided to work with a particular variety or regional strain, a foundation plot is established consisting of 500 to 1,000 plants. The plants are spaced 3½ feet apart each way. Nothing is done during the first season except to score the plants for desirable characteristics on the basis of general appearance. First year notes on sweet clover foundation plots are valuable mainly as supplementary information on plants which may be selected for selfing the second season. In many cases, appearances the first season give little indication of what to expect the second season.

In the second season the plants are frequently observed up to the time that first blossoms appear. Each time that observations are recorded, the most desirable individuals are noted and finally a certain number of them are selected for selfing. This is accomplished either by covering a few racemes on each plant with long, narrow, mineralized tiffany bags or by covering the entire plant with a large cotton cage. In the case of foundation plants, the latter method is preferred both because a much larger quantity of seed can be secured by this method, and also because there is a little less danger from the introduction of foreign pollen. Before being covered, each plant is very carefully inspected for open flowers and all such are removed.

The next spring, 50 seedlings from each mother plant are grown from selfed seed in the greenhouse. These are transplanted into the field about June 1st and spaced so as to leave plenty of room for development. These are first generation selfed lines. Each line is inspected closely toward the end of the first season, special attention being paid to uniformity and desirability of plant type within each line, including colour of foliage. We have found that plants with dark green foliage have an advantage over those with light green foliage, especially with respect to disease resistance.

During the second season's growth and previous to flowering, these selfed lines are inspected several times. Growth is very rapid, and repeated observations are necessary for accurate comparisons. In general we prefer those families which show an absence of winter injury, quick early growth in the spring, dark green foliage, semi-dwarf plants with numerous, fine, leafy stems and, recently, self-fertilization. This last characteristic is determined by examination of a few flowers on each plant by the method described in recent papers (**5, 6**).

If a first generation selfed line does not approach rather closely to the desired type and is not fairly uniform in that respect, it is discarded at once. About 95 per cent of the lines are discarded at this stage for one reason or another, and the other 5 per cent. retained. Each year a new group is

must be watched closely. When the insects become sufficiently numerous to cause serious damage, the cage is covered with a canvas tarpaulin and one sheet of nicotine paper burned inside. After 15 minutes the canvas is removed. One treatment is usually sufficient in each season.

Lucerne (Medicago sativa).

In our investigations with lucerne, the results of breeding from an economic standpoint have been regarded as secondary to a study of the effects of inbreeding and its possibilities as a practical method of improvement. With this in view we began with a considerable number of strains, each with distinctive characteristics and all of them sufficiently hardy to withstand the rigorous winter climate of Western Canada. Through each of four generations, the selfed lines were tested for yield of forage and seed in comparison with original material, and the general effects of inbreeding were noted. Having completed a study of this kind through four generations over a period of ten years, in which all the lines of descent were maintained, except a few which were automatically eliminated through failure to reproduce, we are now giving more attention to the most vigorous inbred families in an effort to find out whether their productivity will be maintained in later inbred generations, and how the latter may be utilised to greatest advantage.

The most conspicuous result of inbreeding in lucerne was seen to be a marked reduction in vigour. With most of the original stocks this could be observed up to and including the fourth generation. Contrary to expectation, the decrease in yield was greater in the second than in the first generation and in some lines of descent the greatest decrease occurred in the third generation.

Loss in vigour of growth was accompanied by a very marked decrease in seed production. In the second generation this amounted to 30 per cent. as compared with the original material and it was considerably less than that in later generations.

About five per cent. of the 4th generation selfed lines are approximately as vigorous as the standard variety from which they originated. All of these, except one, belong to a single line of descent. At least two out of four of these vigorous selfed lines give promise of good seed production. All of them are quite uniform and of good forage types. This is the one hopeful feature to date with this method of breeding.

From the results of our studies, therefore, it seems probable that selfed lines can be isolated with sufficient vigour to be utilized as improved varieties if they show special merit of agronomic value and the plants will have the unique distinction of being uniform with respect to the desirable qualities which characterize the variety as a whole.

We are also investigating the possibility of utilizing selfed lines in artificial crosses and the development of synthesized varieties.

Selfing is effected by bagging the racemes with half-pound Kraft paper bags. Crossing is accomplished by emasculating with a needle just before the anthers are ripe and pollinating from two to five days later.

The results of inbreeding have not been impressive as a practical method of improvement. Because of the nature of the experiment, it was not possible to investigate a sufficiently large number of inbred lines to give an adequate idea of its possibilities. Had we been working solely for economic results it would have been possible to accomplish more with much less effort. The method of inbreeding with selection, however, does offer special advantages for certain purposes. It will undoubtedly play a part in any comprehensive breeding project with lucerne.

Fig. 1.—Alpha sweet clover. An improved dwarf fine-stemmed leafy type.

Fig. 2.—Selfing a strain of lucerne with ½-pound Kraft paper bags.

To face page 10.

Fig. 3.—Lucerne breeding nursery. The view is across the row plantings of inbred lines.

Fig. 4.—Self-pollinating cotton cages enclosing selected plants in the brome grass breeding nursery.

To face page 11.

Brome Grass (Bromus inermis).

Bromus inermis and *Agropyron tenerum* are the two most popular grasses in Western Canada. The latter is a native species which is used extensively for hay. The former is a much better pasture grass, but it is grown widely for both hay and pasture. Both grasses are adapted to areas of limited rainfall and may be regarded as highly drought-resistant. Brome grass is superior for grazing since the herbage is succulent throughout the season and very palatable.

The chief objection to brome grass is its strongly stoloniferous habit of growth. It quickly becomes root-bound and cannot usually be eradicated without more than a normal amount of cultivation. In the drier parts of the plains area there should be little objection to it on this score, but on heavy soils and where moisture is more plentiful, it does give some trouble.

Because of the highly nutritive and palatable character of the herbage, and because of its adaptation to areas with limited precipitation, we have regarded the improvement of brome grass as very important. The species is notoriously variable in plant type, ranging from dense, leafy plants to those which exhibit excessive growth of stolons and coarse sparsely-leaved tillers. In our efforts to improve this crop we have selected both pasture and hay types, always with dense leafy herbage and restricted development of stolons.

The method employed has been that of continuous inbreeding with selection within the self-fertilized lines. One hundred families are grown in each generation and about 40 plants per family. Seedlings are grown in the greenhouse and transplanted into the field. The plants mature the following season and selected individuals are self-pollinated. Each generation, therefore, requires two years. Four generations of plants have been studied.

Progress in the breeding of brome grass to date has been very satisfactory. The project is not yet beyond the single plant stage, but interesting hay and pasture types have been developed which are very promising and quite uniform. These will be multiplied and tested as improved forage strains in comparison with commercial seed. Special tests will be made to determine whether these improved strains are amenable to eradication because they are less creeping.

The method employed in selfing brome grass is to enclose the entire plant in a cotton cage, $3\frac{1}{2}$ x $3\frac{1}{2}$ x 6 feet in size. The cotton is supported by four 2 x 2 inch wooden stakes which are held apart at the top by pieces of lath. Cages are made of heavy cotton sheeting similar to that used at Aberystwyth as described and illustrated in Bulletin No. 3.* The material which we used has a few less threads per inch, but it was found possible to increase the density considerably by having it shrunk with hot steam at a laundry.

As soon as the plants begin to head, the stakes are erected so as to have them in readiness for the cotton covering on short notice. The anthers may be exserted as soon as the heads have emerged from the sheath, or flowering may be delayed for two or even three weeks, depending on soil moisture conditions. It is not advisable to cover the plants until necessary, but the cages must be put into place on the first indication that flowering is about to occur. As an additional precaution against cross-pollination, every uncaged plant in the field is cut. Under these conditions it would be necessary for pollen to escape from one cage and enter another before stray pollen could contaminate the breeding material.

Other methods of protection against cross-pollination have been tried, but without success. The heads have been covered with bags or envelopes made of glassine, parchment, and paper, both with and without ventilation, but no selfed seed was obtained, nor have we been successful in securing selfed seed in the greenhouse, although the plants develop and appear to flower normally. It would seem that the large cotton cages used out-of-doors have a special advantage.

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*Bull. No. 3. Imp. Bur. Plant Genetics: Herbage Plants, June 1931.

As to the effect of inbreeding on vigour of growth in brome grass, casual observation shows clearly that some selfed lines are much less vigorous than others. Indeed some of them are considerably reduced in vigour, but the percentage of these is relatively small. The great majority of selfed lines do not appear to be reduced in vitality. Yield of herbage, however, as determined from single plants, is almost directly proportional to the diameter of the plants and this, in turn, is determined by the spread of stolons. Fortunately, those plants which are restricted in spread of stolons invariably produce a dense growth of tillers and are usually very leafy types. In these respects they are in sharp contrast with the widely-spreading stoloniferous plants.

With respect to amount of self-fertilized seed, plants vary greatly from those which set no seed at all to those which are heavily seeded. The factors responsible for this result have not been determined. Great differences have been found on occasion between plants in the same selfed line, even though a conscientious effort had been made to effect self-pollination by shaking the plants when the anthers were exserted, each day during the flowering period.

The cotton cages are allowed to remain on the plants until most of the seed is ripe. This is necessary as a protection from the wind and loss of seed by shattering. The ripe heads are harvested and placed in paper bags to dry. Later each spikelet must be carefully examined in order to secure the seed, which is often very scarce and a little difficult to find. With the majority of plants, however, sufficient seed is obtained to reproduce the next generation.

Slender Wheat Grass (Agropyron tenerum).

Slender wheat grass is a native species of bunch grass which has been grown extensively for many years in the three prairie provinces of Western Canada. Locally it is called western rye grass.

Slender wheat grass presents a wide variety of forms from which to select desirable forage types. As a cultivated crop it is adapted essentially to the production of hay rather than pasture. In certain natural range areas it forms an important constituent of the herbage.

Slender wheat grass is mainly self-fertilized, thus making the problem of improvement by selection comparatively simple. A large proportion of the progenies from single selected plants are uniform in plant type and there is no measurable reduction in vigour from the use of inbred seed. The method to be followed, therefore, is to select individual plants and test their progenies.

While the species is mainly self-fertilized, there is ample indication that the possibility of natural crossing should not be disregarded. Not all of the single plant progenies breed true and we have had the experience of finding a small proportion of off-types in the progeny of families which we had reason to believe had been true-breeding. For these reasons our practice at present is to test and multiply all promising lines by using self-fertilized seed.

Self-fertilized seed may be obtained very simply by covering the heads with paper bags. Almost any kind of bag or selfing envelope is satisfactory for this purpose.

A full account of an improvement project with slender wheat grass has been published (7). Seed was taken from about 150 selected plants and seeded in rod rows with check rows of commercial seed. Rod rows of spaced plants of the same material were grown also at the same time. Yields were taken from the seeded rows for three successive seasons and on the basis of these, together with our general observations, it was possible to select 20 of the best for further testing. These were seeded in rod rows replicated eight times. Six of the plots were cut for yield of herbage and three were harvested for seed.

On the basis of the replicated plot test, six strains were chosen for further study. These were compared in small drilled plots, seeded as they would be in the field. The best procurable commercial seed was included with the improved strains as a check and the plots arranged in a Latin Square.

Fig. 5.—Brome grass breeding nursery, showing a uniform desirable strain (centre) and a coarse strongly-creeping strain (right).

Fig. 6.—Breeding nursery of slender wheat grass (*Agropyron tenerum*).

To face page 12.

The random variability in the Latin Square test was remarkably small, being represented by a probable error of 1½ per cent. One strain exceeded all the others in yield very considerably, with definite indications that the differences could not be due to chance.

Subsequent tests of the improved strain, seeded in larger plots, in comparison with the best grades of commercial seed, showed that the former exceeded the latter by about 35 per cent. in yield of both hay and seed. Such a result is tangible evidence of the improvement which may be effected in this species by plant breeding methods.

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NOTE.—Figs. 1—6. Photographs taken in the breeding plots of the Dominion Forage Crops Laboratory, University of Saskatchewan, Saskatoon, Sask., Canada.

II.—THE BREEDING OF *CICER* AND *CAJANUS*

BY

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The improvement of these two crops in India has up to the present been the result of selection. Hybridization was commenced only a few years ago and the results of this work have not yet been published. Both crops are widely grown in India and selection work in each case commenced with the collection of samples of seed from all over the country through the local agricultural departments. Each seed sample was sown in Pusa and the characters of the plants noted. Selfed seed was taken from selected plants and the work continued in this way from year to year until as many different types as possible were obtained in a true breeding condition. Promising types are then selected for yield trials against the ordinary mixed country crop and, if found superior in yield or quality, seed of these types is multiplied and distributed to local agricultural departments for dissemination among cultivators. The above brief outline describes the normal process of improvement by selection for almost all the crops which have been studied at Pusa and the following notes deal with special difficulties in the technique of breeding *Cicer arietinum* and *Cajanus indicus*.

Cajanus indicus Spreng.

Cajanus indicus Spreng. is a leguminous crop which is widely cultivated in India as a food for man and beast and is a valuable rotation crop for restoring nitrogen to the soil. The plant grows as a tall bush and is generally sown early in July with the first rains of the monsoon and harvested in the following March or April. The seed rate is 10lb. per acre and yields as high as 2,000 lb. per acre have been obtained, but the average is probably about 1,200 lb. per acre.

POLLINATION. Flowering extends over a very long period. The first flowers generally appear at the end of November or early in December, and flowers are still being produced in March when the bulk of the crop is ripe. They are borne in racemes and in some types are clustered together, forming a terminal panicle. The anthers surround the stigma and burst in the bud stage a day before the bud opens. Self-pollination, as in many other plants of this order, is the rule, but crossing takes place freely, the plants having many insect visitors. Seed setting is very largely dependent on the weather and is favoured by bright sunny days. Damp and cloudy weather has a very adverse effect on seed setting, the yield of seed in Bihar being greatly reduced if dull weather prevails in January and February. An estimate of the amount of natural crossing was made by Howard and others, who state that the proportion of heterozygotes in the progeny from unbagged plants of a definite pure line was 2.25 per cent. when the estimation of heterozygotes was based on the characters of the flower. If the characters of the seed are also considered the proportion rises to 5—6 per cent. Further observations in the seasons 1927-28 and 1929-30 made on ten different cultures grown on a large scale showed that the percentage of natural crossings based on the characters of the flower ranged from 0.15 to 7.59 per cent. The estimation of the proportion of heterozygotes depends upon the ease with which the heterozygote can be distinguished from the type of the parent culture. This was most readily done in a type in which the back of the standard is pure yellow without any red marks and in which this type of standard is recessive to forms having red marks on the standards.

PERCENTAGES OF NATURAL CROSSING IN RAHAR TYPES.

Type.	Percentage of crossing, 1927-28.	No. of plants, 1927-28.	Percentage of crossing, 1929-30.	No. of plants, 1929-30.
M. ..	2.6	6070	7.59	580
F. ..	0.14	3600	0.59	851
E. ..	6.7	2238	6.73	1427
C. ..	0.99	10249		
H. ..	1.5	4720	1.66	1085
K. ..	2.9	3980	6.83	600
212 ..	0.09	5430		
D. ..	0.32	18093	2.07	770
A. ..	0.56	8473	1.04	1723
G. ..	0.16	5618	0.68	881

This experiment was carried out with seed which had been obtained in 1926-27 from cultures growing in lines side by side with other cultures. The conditions were, therefore, exceptionally favourable for cross-fertilization.

SELF-FERTILIZATION. Plants set seed readily under muslin bags. For this purpose it is the practice at Pusa to use a muslin having 70 meshes to the inch. Bags may be constructed of any size, but for *Cajanus indicus* a diameter of about 18 ins. is used. The muslin is sewn into a sleeve of 18 ins. diameter and about four feet long. Three rings, 18 ins. in diameter, of split bamboo are sewn inside the sleeve, two about 12 ins. from each end and the third ring in the middle. One end of the sleeve is then sewn up so that it terminates in a cone. The open end of the sleeve is then placed over the branch to be bagged and tied securely on to a layer of cotton wool which has been attached round the stem. The closed end of the bag is attached to a string which hangs from a gallows. If a number of plants have to be bagged, the provision of a gallows for each plant is troublesome and costly as the gallows are made of wood or bamboo and rarely last more than one season. We have found it more convenient to sink light iron posts at the edges of the plot and stretch wires to which bags can be attached across the whole plot. It is generally possible to secure a specimen of a phenotype which it is desired to bag sufficiently close to a wire to permit of the bag being attached to the wire.

CROSS-POLLINATION. This presents little difficulty as the female parent readily stands emasculation. Emasculation is carried out in the young bud about two days before it is due to open and pollination is done on the day following emasculation.

Cicer arietinum L.

Cicer arietinum L., Indian gram, is an important cold weather crop in India. The crop is sown in northern India about the end of October and harvested at the commencement of the hot dry season in April. The sowing rate is 30 lb. per acre and the average yield is about 1,200 lb. per acre, although returns as high as 2,843 lb. per acre have been obtained at Pusa. The grain furnishes an important food for man and cattle and the plant serves as a restorative of nitrogen to the soil.

POLLINATION. About 85 types of this crop have been isolated at Pusa. Flowers are borne singly on short peduncles, but in a few types flowers occur in pairs. Flowers open during the day from about 9 a.m. and close a little before sunset, as a rule they open again on the next morning and finally close in the afternoon. Flowering continues in a plant for about one month.

The anthers are in a group round the style a short distance below the stigma ; before the flower opens, dehiscence takes piace in the bud, followed by an increase in length of the filaments, which carry the anthers above the stigmas. Self-pollination, is, therefore, the rule in this crop, but insects visit the flowers in large numbers and cross-pollination may occur ; it appears, however, to be very scanty.

Damp cloudy weather is an important factor, the seed setting under these conditions being only about 25 per cent. of that which takes place in bright sunny weather.

SELF-FERTILIZATION. The flowers are very susceptible to bagging and it is almost impossible to get a plant to set seed if paper or parchment bags are used. This is due to the fact that the moisture content of the air inside the bag is too high and under these conditions the buds do not open and the flowers drop off. Under favourable weather conditions it is possible for seed to set under muslin bags, but at Pusa pure types were obtained without the use of bags on the plants.

CROSS-FERTILIZATION. Crossing is difficult in this plant as flowers are very susceptible to mutilation. The removal of anthers from the female parent must, of course, be carried out in the bud stage and it is difficult to do this without damaging the corolla. Very slight damage to the flower causes it to wilt and drop off.

STERILITY. Among the factors which affect the yield of grain is the amount of sterility, *i.e.*, the proportion of pods without seeds and with more than one seed. This form of sterility is quite distinct from that which depends upon the failure of pollination due to unfavourable weather and varies markedly in different types. Types which are high yielders generally possess a low percentage of sterile pods and a high proportion of pods with two, or more than two, seeds.

CHROMOSOME NUMBERS. The large seeded " kabuli " grams are very different in appearance to the " desi " varieties which are commonly grown in India. A cytological examination has suggested that the chromosome number in " kabuli " types is 16 while in " desi " types it is only 14. In one of the " desi " grams isolated at Pusa—Type 22—a giant mutant has appeared. This mutant has bred true to its own type from its first appearance in 1927 and appears to possess 16 chromosomes whereas Type 22 has only 14 chromosomes.

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Shaw—Bagging of *Cajanus indicus*.

To face page 16.

III. THE IMPROVEMENT OF *PHALARIS* SPECIES BY SELECTIVE BREEDING.

BY

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INTRODUCTION.

Over a large area of country in Southern Australia, conditions of winter rainfall and summer drought prevail. The principal herbage plants of this area are annuals which ripen seed in advance of the summer, or perennials capable of withstanding the period of stress in a vegetative state.

The *Phalaris* group contains a number of species adapted to this type of climate, but only one of them (*P. tuberosa*, syn. *bulbosa*, *nodosa*, *stenoptera*, etc.) has been of value for the establishment of permanent pastures under conditions of summer drought. The annual species, *P. minor*, *P. canariensis*, *P. paradoxa*, *P. angusta*, and *P. lemmonnii* are capable of drought-escape as a result of vigorous seed-production during late spring, but none persists under grazing conditions. *P. minor* is the most aggressive of these and occurs naturally throughout the cultivated areas of South Australia. The perennial species *P. tuberosa*, *P. coerulescens* and *P. arundinacea* persist vegetatively, but the last-named is dormant during the period of winter rains and produces little feed in summer—its normal growing season. *P. tuberosa* may be classed as a proven species for permanent pasture in the Adelaide environment, whereas *P. coerulescens* is still in the experimental stage.

IMPORTANCE OF PHALARIS TUBEROSA TO SOUTH AUSTRALIA.

At the Waite Institute, *Phalaris tuberosa* has been particularly successful in trials carried out with more than 300 pasture species during the last six years. It possesses the degree of drought-resistance, persistence, and productivity necessary to justify its use in the improvement of sown pastures in Southern Australia. Of particular value is its vigour during the autumn and early winter months, when feed production is ordinarily at a low level. The growing season of this species extends over a wider period of the year than that of the usual pastures employed.

Although *Phalaris tuberosa* has been grown in South Australia since 1906, less than 200 acres of the grass have been established in the State, and of these more than 100 acres are on one farm. Nevertheless, it is still to be found persisting in small plots established by the Department of Agriculture in different parts of the State more than 20 years ago.

CONFUSION BETWEEN PHALARIS TUBEROSA AND PHALARIS MINOR.

Unfortunately, much of the commercial seed sold in the past was the inferior annual *Phalaris minor*, or contained a high proportion of this species. The " seeds " of the two grasses are somewhat similar in appearance, and although typical " seeds " of each are distinguishable, a gradation of intermediate types exists. Moreover, to the layman, the two species are not widely dissimilar in general appearance, and the resulting confusion has probably been responsible in large measure for the present restricted use of the perennial species.

Owing chiefly to difficulties experienced in obtaining pure *Phalaris tuberosa* seed, little progress was made with this grass until recently. During 1929, 20 samples were grown at the Waite Institute,

and 10 of these proved to be *Phalaris minor*. An investigation was then undertaken with the object of finding reliable means of detecting the annual species in seed samples and in the field (1). The presence of pink root tips has proved to be a positive identification character for *Phalaris minor*, and the use of this test enables the annual species to be detected with certainty a few days after the receipt of a seed sample. During 1930 and 1931 efforts have been concentrated on the selection of desirable pasture types of *Phalaris tuberosa*.

Main Characteristics of Phalaris Tuberosa.

Phalaris tuberosa is a long-lived perennial from two to seven feet in height at maturity, characterized by the possession of a woody rootstock underground, with widely spreading tillers, the basal portions of which are stoloniferous. The vegetative growth is leafy and succulent, rather resembling that of *Phleum pratense*. Towards flowering, reserves are transferred to the lowest two or three internodes, which become bulbous. The panicle is spikelike, typically long and tapering. The root system is encased in a fibrous covering and has been traced through subsoil clay at the Waite Institute to vertical depths ranging from 42 to 58 ins. During the arid summer period the plants remain in a dormant or semi-dormant condition.

Material and Methods of Study.

The *Phalaris* material employed has been collected chiefly in South Australia, but samples have also been obtained from Palestine, Morocco, Southern France, California and Oregon (U.S.A.), New Zealand, Victoria, New South Wales, and Western Australia.

An examination of this material has indicated that habitat selection can be of little value at the present stage. Relatively few habitats are available compared with those from which ryegrass, cocksfoot, and other widely cultivated species may be collected, and the conditions of these habitats are comparatively uniform. Lines from different sources in general show greater variation within the line than as between line and line, although some lines contain a greater proportion of desirable types than others. This may also be due in part to the relatively short period over which this species has been cultivated, allowing small opportunity for the time factor to operate. *Phalaris tuberosa* has therefore been studied principally under single plant conditions, and single plant selection has so far been exclusively employed.

Variation in Phalaris Tuberosa.

Individual plants display considerable variation in type, and may be arranged along a scale with comparatively dense leafy forms possessing truly creeping rhizomes at one end, and plants with exposed crowns and a few coarse stemmy tillers at the other. At the top end of the scale are types which resemble the actively creeping *P. arundinacea*, whereas some of the erect types approach *P. minor* in appearance. The occurrence of " perennial *P. minor* " types lends additional interest to the range. These are definitely perennial, but in common with *P. minor* are erect with papery green leaves, exposed rootstocks, and pinkish root tips. The head type is intermediate between standard heads of *P. tuberosa* and *P. minor*, and the plants are stoloniferous, the lower nodes producing roots. Dr. I. F. Phipps has found " lagging " chromosomes in this type.

Adopting Stapledon's system of classification (2) for the various types of Cocksfoot, the majority of *Phalaris tuberosa* plants grown at the Waite Institute fall into " cups " and " lax hay " groups, with " dense hay " and " tussock " forms comparatively rare, and the extreme pasture types practically non-existent. Under perennial hay conditions individual plants exhibit a high degree of persistency and drought-resistance, and in the main seed-production is satisfactory. Selection aims at increased density, leafiness and vigour, with greater adaptability to sward conditions. A number of types have been selected in which the above characters are combined with reasonable seed production, and breeding tests are being carried out on these plants under " selfed " conditions. In certain of the selected types, definite rhizomes are present.

Technique Employed.

Seeds are sown regularly during autumn in boxes 20 ins. x 15ins. x 3½ins., containing steam-sterilized soil, and the seedlings are transplanted during early winter in rows 2½ links apart with two links between the plants in the rows. It has been found, however, that the plants when fully developed are rather crowded under these conditions, and a spacing of 4 x 4 links (=32ins. x 32ins) will probably be used in future. Units of 50 plants in two rows have been generally employed, with every tenth row a check.

Selected plants are selfed by means of vegetable parchment bags 15ins. x 8ins., enclosing a unit of five heads per plant. Balloons of cotton fabric previously examined microscopically for closeness of weave have also been used in certain cases, the unit here being 20 heads per plant. Seed-production is measured in terms of numbers and/or weight of seeds per inflorescence, owing to the great difficulty of determining the number of spikelets in the head.

Controlled inter-crossing has not yet been employed in connection with strain production. Inter-pollination in isolation of plants selected first for type and then on the basis of their selfed and out-crossed progeny will be attempted for the first time in 1932. On the other hand, as Jenkin has already shown (**3**), hand emasculation of *P. tuberosa* and *P. arundinacea* may be readily carried out by the continuous removal of the anthers between partial exsertion and dehiscence, and this method is at present being employed in attempts to inter-cross in pairs *P. arundinacea*, *P. tuberosa*, and *P. minor*. The two perennial species were successfully inter-crossed as early as 1921 by Jenkin (**3**.)

Preliminary Results Obtained on Selfing.

The study of both parent and first generation plants from the same lines was first possible in 1930, when 93 individual plants were bagged, with the production of 30 second generation lines and an additional 44 first generation lines. 20.4 per cent. of the total selfings have yielded no seed, of which 5.4 per cent. also yielded no " open " seed. Seed yields on selfing, during both 1929 and 1930, demonstrated low self-fertility in *Phalaris tuberosa*, the production under bagged conditions varying from one seed per five heads to a maximum of 0.194 gms. per head, with a mean value of 0.13 gms. or six seeds per head, whereas the yield from the open heads of the same plants varied from 0.012 gms. to 1.128 gms. per head, with a mean value of .188 gms. or 90 seeds per head. No correlation appears to exist between seed-production and size of head, type of head, or the ratio of its length to its breadth. In general, the early, erect and stemmy types set seed more abundantly than the later, denser, and more leafy types when open-pollinated, although a number of the most promising types for pasture purposes are well above the average in seed-production.

Inter-bagging heads from neighbouring plants increases seed-production considerably above that of either parent when selfed, but does not approach that of the " open " heads of the same plants.

Selfing decreases the vigour of the progeny, the reduction varying from scarcely apparent to fairly marked, depending on the plant selfed. On the other hand, no decrease in seed viability has been obtained. Selfing has in a number of cases resulted in a high degree of uniformity among the first and second generation progeny.

In the case of the two annual species *P. minor* and *P. canariensis*, anther dehiscence normally occurs prior to exsertion, and self-pollination is the rule. The seed yields of these species are as high under bagging as under " open " conditions, and no reduction in vigour takes place.

Concentration on Perennial Types.

In 1931 the material for observation and breeding purposes was confined to *P. tuberosa*, with four lines of *P. arundinacea*, and one of *P. coerulescens*. The annual species have been discarded because of their unsuitability for pasture purposes.

P. coerulescens was first subjected to selfing in 1931. The plants are tall (approximately five feet at maturity), erect, dense, and fine-leaved with rather long inflorescences. Flowering takes place several weeks earlier than in any of the *P. tuberosa* lines and coincides with that of *P. minor*. In common with *P. tuberosa*, this species possesses an underground rootstock with stoloniferous tillers.

TENTATIVE ESTABLISHMENT OF ISOLATION GROUPS FOR SEED-PRODUCTION.

It is proposed to establish tentatively during 1932 seed-production islands composed of parents vegetatively propagated and selected on the basis of their own characteristics, and the performance of their progeny under selfed and open-pollinated conditions.

Although it may be many years before anything approaching a pure strain will be produced, considerable improvement over commercial lines can probably be obtained by the immediate multiplication in isolation by distance of the best material at present available, involving the removal of inferior types occurring in each successive generation. At the same time, some 60 lines are being carried on under selfed conditions, with concentration on the purification of types selected for density and leafiness. Several lines will be brought to the third generation, and additional data obtained on seed production vigour and uniformity of type under a programme of continued self-fertilization.

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IV.—BREEDING TECHNIQUE FOR *PENNISETUM TYPHOIDEUM.*

BY

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Bajri, *Pennisetum typhoideum*, is an important cereal crop of the drier regions of the Bombay Presidency. It is a monsoon crop, sown on unirrigated lands during the latter half of June or during July. It occupies the ground for three to four months. This crop shows great response to even slight variations in environmental factors. The variation in yield of grain is from 400 lbs. to 2,000 lbs. per acre depending on soil, rainfall, variety, etc. The crop is grown mostly for its grain which is made into unleavened bread, but the straw is also used as fodder.

Inflorescence.

The inflorescence is a compound spike with an unbranched, more or less tapering central axis. On this are arranged spirally a number of rachillae, each carrying a group of spikelets, usually two, enclosed in a whorl of bristles. Sometimes these bristles are very long, when they resemble awns. Great variation is found in the thickness of the central rachis and the length of the rachillae in different earheads, but it is generally observed that these characters are negatively correlated, so that a thick rachis generally bears shorter rachillae. There is also considerable variation in the tapering of the central rachis and the length of rachillae on different parts of the same spike. Generally it is found that the longer rachillae are on the central region of the earheads while the shorter ones are at the apex. The variation in thickness of the central rachis, its tapering and the decrease in length of the rachillae towards the apex of the spike, determine various shapes of earhead, the chief among them being the cylindrical, conical and spindle-shaped. There is also great variation in the number of rachillae per unit of surface area from spike to spike and in different parts of the same spike.

Spikelet.

As already mentioned, each rachilla bears a group of spikelets, generally two, enclosed in a whorl of bristles. Rachillae bearing one or three spikelets are not uncommon while those bearing four spikelets are rare. This variation may be found from spike to spike or in different parts of the same spike. The spikelets are ovate in shape and have a very short pedicel. The empty glumes are much reduced. Above these are the two inner glumes which enclose between them two flowers of which one is hermaphrodite and the other staminate. Variation is found in the presence or absence of the staminate flowers in the spikelets, but this depends to some extent upon the number of spikelets in the rachilla. The stamens are three with filiform filaments and versatile anthers. The stigma is bifurcated and is covered with stigmatic hairs.

Flowering and Pollination.

The emergence of the spike on the main stem follows rapid elongation of the peduncle and the internodes below, within five to ten weeks after the date of sowing. Two or three days after the emergence of the spike, the styles begin to protrude and require about two days to reach their maximum length. They remain fresh for about a day and begin to dry rapidly. Only then do the anthers begin to appear from the hermaphrodite flowers. The first flush of anthesis is complete in about two days ; about a day later, the anthers from the staminate flowers protrude and thus there is a second flush of anthesis for two days more. The dehiscence of the anthers occurs at any hour of the

day, but the rate of dehiscence is much slower during early morning than after sunrise. Clouds of pollen, floating in air, are carried away easily by wind. Apart from this agency, a small bee (*Apis florea*) visits the flowers and collects pollen. Other insects, viz., flies, beetles, grasshoppers, also visit the spikes and are also probably responsible for transferring some pollen from plant to plant.

In about eight days from the emergence of the spike, the grain setting begins, but is well marked only after twelve to fifteen days. A week later the grains become fully developed, but are milky in colour. During the next week they begin to change their colour from white to pale or deep blue. It takes about a month for ripening of the grain from the day when the fertilization is complete.

SELECTION.

Since the plant is so markedly protogynous, it is naturally cross-pollinated, and this makes it very difficult to get homozygous progenies, even after generations of selfing.

The susceptibility of the plant to variations in environment also tends to blur the results of selection.

As yield of grain is our primary object of breeding, we have been concentrating more on those characters which are associated with yield. The following characters we believe to be of importance—

(1) *Length of the earhead.*
(2) *Diameter of the earhead.*
(3) *Shape of the earhead* (depending on length, the maximum diameter and reduction in diameter towards the base or apex).
(4) *Compactness of the earhead* (the number of rachillae, spikelets per unit surface area, length of rachilla, thickness and tapering of the central rachis).
(5) *Size of grain.*
(6) *Tillering.*

In addition, attention has been paid to earliness or lateness.

All but the last of these characters are more or less related to yield and are also heritable. The actual process of selection has been as follows :—

All the earheads collected from different localities are grown in duplicate lines and at the end of the season those are eliminated which are distinctly useless in both samples. Among the remaining lines, promising plants from promising lines are bagged. It is desirable to select for individual characters in the first instance. The behaviour of such individual characters in succeeding years should be closely watched. To take a concrete example let us consider earhead length, which is definitely correlated with yield. In selecting lines for length of earhead we should arrange the selected selfed earheads in a serial order according to their measurements, and sow them in lines of about 50 feet length, the lines being in duplicate and the order of lines being reversed in the duplicate as shown below.

Lines arranged according to maximum earhead lengths—

1, 2, 3, 4, 5, 6, 7, 8
8, 7, 6, 5, 4, 3, 2, 1

The same process will have to be followed for the earhead diameter and grain size. For the other characters which are rather less easily defined, such as earhead compactness or flowering time, it is desirable to follow the same procedure, to give the environment the chance to do its worst, and so make more clear what is really heritable.

In this way it should be possible to obtain pure lines differing in morphological characters. Apart from this, in selection it is necessary to judge the yield performance of progeny.

Other characters that appear to be heritable are :—

(1) *Degrees of hairiness of stem nodes, leaf sheath and lamina.*

(2) *Degrees of red colour on the stem and nodes.*

BAGGING.

Though bajri is distinctly protogynous, self-pollination by bagging the inflorescence is possible and the seed is found to set well within the bag. To ensure success in selfing, it is necessary to bag the inflorescence at a very early stage. The approaching emergence of the spike is indicated a day or two earlier by the flag stage. It is better to bag the spike just when it is out of the flag. In doing so it is necessary to cut off the flag lamina carefully in order to put on the bag properly. The bag should be first loosely tied to allow free elongation of the peduncle of the spike and after a day or two it should be fastened tightly. The bag to be used may be of very fine muslin, so that the chances for the entrance of foreign pollen through the meshes should be low. A bag of thin, glazed semi-transparent paper known in the market as butter paper or oil paper may also be used.

If heavy rains are expected it is advisable to protect plants, especially those that are bagged, by tying them to wooden or bamboo supports. The paper-bagged inflorescences should be again enveloped by other paper-bags to avoid damage and soaking. These envelopes should be removed after the rain is over.

A side-effect of bagging is that stigmas remain fresh for a longer period than in the open condition. This was especially noticeable in the hot weather when bajri (grown out of season for getting an additional generation) set seed *only* in bags.

Bags (either muslin or paper) should be removed after about eight to ten days when grain setting is observed.

CROSSING.

The interval between the ripening of the stigma and the bursting of the anthers in bajri may be utilised in effecting mass cross-pollination. The method is as follows :—

The seeds of the types selected as parents are sown in alternate rows, the distance between the rows being four feet. The stages of flowering should be so adjusted by timing the sowings that one parent is in the stigma stage while the other is putting forth its anthers. The heads of the variety used as a male parent are bent towards the heads of the female parent and the pollen dusted direct from them on the stigmas of the female parent, three times in one day.

As the season has a great effect on the proper development of the plant and the time of flowering, it is necessary to water the plot in case there is want of sufficient moisture in the soil.

For individual cross-pollination we have to take advantage of the fact that the interval between the appearance of the style and the anther is greater in the lowermost region of the spike than in the other. The necessary procedure will therefore be to cut off the upper two-thirds of the spike before any styles appear, and bag the spike, preferably with a good paper bag. After two days, when the styles are seen, pollen from a previously bagged inflorescence should be dusted on the stigmas. Dusting should be done at least three times in one day to ensure fertilization.

V. METHODS EMPLOYED IN PURE LINE BREEDING OF INSECT-POLLINATED PLANTS: RED CLOVER.

BY

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(Translated and adapted from the Russian manuscript)

INTRODUCTION.

Breeding work with fodder crops has been in progress in the Soviet Union only for some four to five years. Among the thirty chief fodder plants in cultivation, 90 per cent. are exogamous, thus making breeding work more complicated than is the case with cereals. Nevertheless the breeding of good fodder plants is at least of equal importance to that of cereals in the present stage of rural economical reconstruction. Scattered attempts along these lines have been made by separate research institutions and breeders, but certain more doubtful points have yet to be solved.

Having found no literature relating to the breeding of exogamous plants (1), I was compelled in my work with red clover to touch upon the most important problems of methods and technique employed in breeding entomophilous plants. These problems include, in my opinion, (a) the use of insects in breeding, (b) the obtaining and preserving of homogeneous lines. These two questions have been elaborated as regards red clover with a view to the application of the method of pure line breeding, the method which in this case promised to be effective.

But before these problems could be approached, the appropriate form of red clover was necessary. Any form with recessive character could be employed as such material, but forms where the character was either rare or well-marked would be more suitable. When such material was obtained, further work would be possible. In brief, our methods were confined to the following points :—

(*a*) *isolation of a red clover form with suitable recessive character,*

(*b*) *recording the yield and quality of the work done by insect-pollinating agents,*

(*c*) *recording the contamination in pure lines under various conditions of generative multiplication,*

(*d*) *elaboration of methods relating to the persistence and multiplication of separate forms and lines in a pure state.*

(a) ISOLATION OF A RED CLOVER FORM WITH SUITABLE RECESSIVE CHARACTER.

The decision that emasculation of red clover was unnecessary was justified by results obtained at the Nossovka Agricultural Experiment Station (2), where the work was conducted ; trials on inbreeding of clover showed that red clover is almost sterile when voluntarily self-pollinated, as on an average 0.2 per cent. of seed setting, varying between 0.1. to 0.4 per cent. in different years, was obtained. This low rate of self-fertility may be neglected.

(1) At that time, the work of R. D. Williams at Aberystwyth was not known to the writer.

(2) See special note appended.

The final material chosen for the work was white-flowered red clover, recessive as regards this character. Such a type was first isolated from samples bred by Prof. Kolkunov, and later from Nossovka clover. The convenience of the chosen form is that it is rare in the vicinity and the recorded character (white flower colour) is well marked.

Even before the commencement of work with this form, both these factors were markedly manifested under the following circumstances. In 1924, I. D. Rogoza made an attempt to isolate white-flowered red clover at Nossovka. White-flowered plants growing among red clover were marked and allowed to flower freely, after which the seed was collected separately. In 1925, the progeny (1,000 plants) were entirely red-flowered. This was because Nossovka clover has the factor for white flower colour only rarely present, white-flowered red clover being rare. Opposite results may be observed, when seed are gathered from free-growing red clover recessive as regards a character more frequently present, such as absence of leaf marking. This is quite intelligible since many plants without leaf marking are present within every population, and intercrossing is more frequent. The white-flowered form, being very rare, had definite advantages, but also had a fault which to some extent complicated the matter and diminished its accuracy. White-flowered red clover, with a slight tinge of rose, resembles in an early stage of flowering a typical white-flowered plant, later becoming rose-coloured ; although they may still be called white-flowered plants, yet intercrossing produces a number of plants with various degrees of colouration in the flowers. As a consequence of the ignorance of this circumstance, Pure line No. 2, one of the first isolated, consisted not entirely of white-flowered forms, but produced even in intercrossing a relatively large number of plants with white flowers tinged with rose, and a small number of distinctly rose or even red-flowered plants.

This circumstance must be taken into account in further examination of the results. In addition, there were observed cases of the simultaneous appearance of white and red florets on the same plant, which may be understood as clear cases of the white-flowered tendency in normal red-flowered plants, dependent on the conditions of growth. In this connection, in order to avoid possibility of error, plants bearing rose-tinged white flowers were recorded as red-flowered types, although from the common point of view they were white-flowered plants.

(b) Methods of Artificial Cross-pollination.*

For practical breeding by the pure-line method, simple and effective methods were necessary, e.g., a simple method of hybridization was essential at the commencement. In this phase, the use of insects offered attractive possibilities, but it was first necessary to know what insects were suitable for this purpose and under what conditions their application was expedient.

With this object in view, a number of comparative trials on the efficiency of various methods of pollination (match, forceps, or various insects) proved the superiority of pollination by match (the tip of which is conical, with a rough glued surface). The yield from work with forceps was somewhat lower, but was higher than that produced by insects. Common honey bees worked actively ; the yield from their work was low. Therefore, with a view to increasing their productivity, more detailed research with a large number of isolators (cages) with varying numbers of humble bees in each was carried out. Trials of 1929-30 indicated that (1) if the breeder desires to obtain a larger amount of seed under the isolator, it is more advisable to increase the number of plants under the isolator ; (2) when a small number of insect pollinating agents is available, it is more expedient to use a smaller number of insects under each isolator ; (3) when it is desired to cross a small number of plants (two plants in pure line method), it is more advisable to use hand hybridization, especially the match, and to apply insect service only to the F_1 generation, or to hybridize, in the isolator, forms which had been propagated vegetatively.

Elaborating further this problem, a preliminary attempt was made to study the efficiency of various humble bee species. Although no final conclusion can be deduced, owing to the wide range of variation, yet it is possible to state that large long-proboscis species (*Bombus mesomelas*, *B. hortorum*, *B. lapidarius*) are considerably more efficient than the smaller species with short proboscis (*Eucera longicornis*, *Bombus terrestris*, *B. sylvarum.* etc.). This conclusion is confirmed by observations on the work of insects under isolation and in the field.

* This section has, with the author's permission, been considerably abridged, as it deals with work which has to a certain extent been already published.

In order to estimate insect work from the qualitative aspect, we used white-flowered red clover, which naturally produces red-flowered offspring when contaminated by foreign pollen. These trials showed that honey bees are unsatisfactory. Humble bees may be used, especially when they have been captured on plants other than red clover. Bees captured on red clover sowings, carefully washed (3-5 mins.) *and thoroughly dried* (1), may also be used satisfactorily. In this case, the maximum contamination will not be larger than 8-10 per cent., or may be reduced in certain cases to nil.

In the study of the quality of insect work, it was possible of course to adopt other, possibly more accurate methods, such as the recording of seed of one bush in the isolator, or the seed produced by the vegetative offspring (clone) of one bush verified as regards its degree of self-fertility. In the following studies, however, the use of generative multiplication was preferred and for this reason the white-flowered red clover was employed for the experimental fields.

(c) Spatial Isolation and Contamination of pure lines.

In this phase it is impossible to carry on testing simultaneously with multiplication, owing to the contamination produced by cross-pollination when lines are grown in adjacent plots. Multiplication must therefore be carried on in some other way, namely by spatial isolation of lines first multiplied by seed or by vegetative methods. In both cases it is, however, necessary to know what degree of purity may be obtained.

It may be assumed that (1) the further apart the forms under multiplication are, the less would be the contamination, (2) the larger the plots the less would be the contamination and (3) if seed are taken from central portions, the contamination would be decreased. The first two questions have been under study at Nossovka since 1929, while the study of the latter commenced in 1930. The data obtained thereby, with the exception of results relating to the quality of seed from various portions of farmland, are tabulated below :—

TABLE 1. Extracted data of spatial isolation.

Degree of isolation.	Isolated in 1929, recorded in 1930.					Isolated in 1930, recorded in 1931.				
	Size of plot sq. m.	No. of plots.	No. of recorded plants.		Per cent. of contamination.	Size of plot sq. m.	No. of plots.	No. of recorded plants.		Per cent. of contamination.
			Total	Red flowered plants.				Total	Red flowered plants.	
*	—	—	—	—	—	1000	5	162	112	69.0
2.5m.	5	5	16,399	10,289	62.8	30	9	626	77	43.4
5 ,,	350	16	20,438	6,356	32.2	200–300	7	549	93	16.9
50-60 ,,	—	—	—	—	—	50–350	8	1104	139	12.5
100 ,,	50–60	5	10,391	1,198	11.5	—	—	—	—	—
250 ,,	500	1	3,745	695	18.5	150–250	2	442	35	7.9
250 ,,	—	—	—	—	—	500	1	401	71	17.1

* This plot was isolated from three sides only.

As may be seen, the large plot (about 1,000 sq m.) may show up to 75 per cent. contamination, if the clover sowings (in this case ordinary red clover) are directly adjacent. It is also obvious from the table that the multiplication of material in a pure state is an easier matter if the amount of seed

(1) The pollen of red clover which has been wet is viable and may still fertilize ; only with subsequent drying will it die.

available is sufficient for a large area. The increase in size of plots from 5 to 350 sq. m. and from 30 to 300 sq. m., when under similar conditions of isolation from adjacent clover sowings (2-5 m.) reduces the contamination (62 and 16 per cent.). Better spatial isolation is to be preferred to larger plots, as experimental data indicate definitely that similar plots of 50-300 sq. m. at a distance of 50 - 100 m. from clover sowings show a decreasing degree of contamination (30-45 per cent. to 11-12 per cent.). The largest type of spatial isolation under study, namely, 200 m. for areas of 150-200 sq. m., produced a high degree of purity (92 per cent.).

Hence, if the fact that the figures of the table give rather the maximal degree of contamination be taken into consideration, we may conclude that for practical purposes of pure line multiplication, spatial isolation of 100-200 m. or perhaps even 50-60 m. is quite satisfactory. The latter size is acceptable because under the conditions of work at Nossovka, this isolation from the clover sowings was much reduced by the fact that wild clover plants were abundant along the roadsides. The plots under an isolation of 200 m. were under the same conditions, as 10 m. away in one direction and 50 m. in the other was a cemetery with other wild clover plants in flower. Apart from this it may be assumed that the scattering of small plots among sowings of other plants, more especially of those pollinated by the same insect species, under similar conditions of spatial isolation with those above mentioned, would probably decrease the degree of contamination of lines.

It is therefore concluded that the spatial isolation of a relatively small area with 2000-3000 plants, situated at a distance of 200 m. from other clover sowings, under usual farm conditions guarantees the preservation of varietal purity up to 90-95 per cent. Increase in size of plot is counteracted by the increase necessary in varietal testing, and by the increased amount of seed necessary for the increased area and repetitions. It is possible and probably more rational, however, to apply the method of vegetative propagation to certain phases of the work, on such a scale as will not disturb the natural progress of work on multiplication and varietal testing.

(d) Vegetative Multiplication and the Preservation of Cuttings.

Methods of vegetative multiplication similar to those used for lucerne and sainfoin (*Onobrychis*) may be employed with red clover. With clover, however, the taking of cuttings in summer and spring is not suitable. Red clover cuttings, in contradistinction to those of lucerne, would die in the autumn after producing seed, while any survivors would be so weak as to be unable to survive the winter. Further, red clover is not highly winter-resistant and forms necessary for the breeder may disappear.

This problem was studied at Nossovka from two aspects :—

(1) *preservation of life of cuttings planted in field over a period longer than a summer season,*

(2) *preservation of strains and separate plants from winter-killing.*

There are indications from Shatilov and Sophinskaya Stations that red clover cuttings, after giving seed, perish completely during the first year, while some breeders hold that they are biologically incapable of resisting the winter.

Research at Nossovka showed that cuttings from the upper part of the plant, taken as separate nodes from adult plants before flowering, would develop badly. Cuttings of lucerne taken under similar conditions would give 60-90 per cent. of rooting, as compared with 10-20 per cent. in red clover. On the other hand spring cuttings consisting already of several nodes taken from young, not yet completely developed stems, 5-10 cm. in length, root well.

TABLE 2. Red Clover Cuttings in 1929.

Number of cuttings.	Summer cuttings.			Spring cuttings.		
	Lower nodes.	Middle nodes.	Upper nodes.	Lower parts.	Middle parts.	Upper parts.
Planted in greenhouse	105	115	115	262	262	262
Grown in field	5	6	8	191	196	203
Survivals after winter	0	0	1	1	0	4

It may be seen, however, that both summer and spring cuttings, after producing seed, are almost entirely killed off by autumn. Under these circumstances, apprehensions will arise that, if for any reason the cuttings do not produce seed by the autumn of the first year, the breeding work would be in vain and the pure line would be lost.

In this connection it seemed to us that it would be more expedient to establish what conditions are necessary to preserve the life of cuttings for periods longer than a year, and to find methods for the preservation of cuttings against winter-killing. For the solution of the first question the following methods were adopted :—

(1) *prevention of seed setting in cuttings during the first year of growth,*

(2) *effect of photoperiodism on plants derived from cuttings.*

We based these experiments on the knowledge that red clover plants (especially those of late single-cut forms), sown by seed, are weakened by seed-setting and in consquence under the conditions of the Nezhin vicinity are usually killed off even before winter, while those plants which produced no seed continue growth into the winter.

In order to prevent seed setting in red clover, it is possible to apply either mowing or shortening of the day, the latter being preferable. Mowing, a very simple and cheap method in mass work, was adopted in the following form. On planting, the spring clover cuttings were separated into two parts ; one was allowed to flower and produce seed, while the other was cut as soon as it reached the flowering phase. These mowings had to be done 3-4 times during the season, as clover cuttings, especially from the tip of the stem, showed a strong tendency to frequent flowering and seed setting, while their rosettes were badly formed. Nevertheless, most plants produced quite satisfactory rosettes. The effect of cutting during the summer as above described was to increase the survival rate over the winter from 3 to 45.4 per cent. The rosettes of some plants (of different clones) appeared to be vigorous, but above one third of them was weakly developed.

The effect of a shortening day was much more marked. The experiment was carried out with clay pots, 15 x 12 cm. in size, with clone cuttings of the same plants, and produced the following results.

TABLE 3.

Pots.	Total plants.	Total which flowered.	Total producing (in autumn)		Number killed.
			Rosette.	Stem.	
Control	5	5	0	5	4
With 10-hour day.	24*	0	15	8	0

* One of which was lost in summer.

In contradistinction to our previous trials, the pots with plants in this experiment were not covered, but were removed for artificial night into a cellar. Results obtained, however, were similar to those of field trials, the plants forming vigorous rosettes during the period of shortened days.

On being transplanted into the field, they entered winter in a healthy condition. It is interesting to note that cuttings of the plants with the peculiarly cut corollas (described in Bulletin No. 113 from Nossovka) did not form rosettes close to the ground surface during that period. In this type the rosette was formed in the stem axil, and the plants flowered weakly and entered winter in an unsatisfactory state, while the shortened day influenced the production on other types of plants of good lower rosettes, developed apparently from dormant or from adventitious buds.

Therefore it may be stated that mowing of cuttings before blooming and more especially the shortening of the days enable the breeder to prepare the cuttings to some extent for over-wintering. The termination of work at Nossovka did not allow us to follow up the winter resistance of plants derived from cuttings compared with those grown from seed. I assume that this will be similar, that the over-wintering of clover cuttings is quite possible and that the differences between clover and lucerne, for example, in this respect are negligible.

Even in these circumstances, however, the risk of loss of cuttings is large enough, as a consequence of the low winter resistance of clover in general, and we therefore gain but little with the above methods. We gain, of course, when, as a result of unfavourable weather, cuttings cannot produce seed during their first year (*e.g.*, they begin to flower too late) and the subsequent winter is very severe. To foresee the possible seed setting with sufficient accuracy, and consequently to determine if the preparation of cuttings for wintering is necessary, is not now a difficult matter, but to foresee the type of winter conditions is as yet quite impossible. Yet a severe winter will require much more work as regards preservation of cuttings in the field, and even then it may kill off suddenly plots for multiplication and varietal testing and eradicate those valuable forms, further seed of which is not available or has for any reason lost its germinating capacity. Hence other more secure methods for the preservation of plants of interest to us are necessary.

Research at the Nossovka Station showed that the preservation of the life of a variety or of an individual plant for an indefinite period and many questions of vegetative multiplication are easily solved by taking cuttings in autumn and preserving them through the winter.

Tests with cuttings from red clover rosettes in late autumn, when plants had terminated the vegetative period (October or November), and with their preservation under various conditions gave the following percentages of cuttings killed during the winter 1928-29 :—

TABLE 4. Percentage mortality of cuttings (100 in each trial).

Cuttings without leaf blades			Cuttings with leaf.			
Without sand.	Inserted in sand.	Between layers of sand.	Without sand.	Inserted in sand.	Between layers of sand.	Conditions in store-rooms.
68.3	59.2	30.1	86.6	67.6	40.5	Excessively damp
62.1	35.6	6.4	60.2	42.7	10.3	Moderately dry cellar.

The cuttings with leaf-blade showed in general higher mortality ; the best results were obtained with cuttings placed between layers of sand, while the poorest results were from those pooled in loose heaps. Cuttings in layers of sand kept in a moderately dry cellar lost little during winter, and on being replanted in spring with renewed lower extremities into the soil of a greenhouse, developed as well as spring cuttings, although their leaves were discoloured and occasionally decomposed.

There are wide differences between various red clover populations as regards persistence of cuttings, which variations are influenced by phytopathological rather than by genotypical factors. The lower immunity of plants to some root and stem diseases (*e.g.*, *Fusarium* or bacteriosis) has a marked influence on their persistence. Thus killing of some clones of single-cut clover was apparently caused by infection of stem, and consequently of cuttings, by a decomposing agent, while on the

contrary, the high percentage of damage obtained in *T. resupinatum* and *T. incarnatum* is apparently to be explained by their biological peculiarities.

After the winter of 1929-30, the autumn cuttings, with leaf removed, covered with sand and kept in cellars, showed on 24-3-30 the following mortality.

TABLE 5.

Red clover species and strains.	No. of cuttings.		Per cent. of mortality.
	Living.	Dead.	
Single-cut red clover (*Trifolium pratense* L.)			
1. Tula strain from isolated field	186	186	100.0
2. „ „ „ varietal testing plots	113	80	41.4
3. „ „ „ isolator No. 1.	90	14	13.4
4. „ „ „ „ „ „	88	20	18.5
5. Winter strain from field spatially isolated	49	21	30.0
Twice-cut red clover (*T. pratense* L.)			
1. Nossovka strain from isolator field	160	21	11.6
2. „ „ „ varietal testing field	302	77	20.3
3. White flowered strain from fields spatially isolated	232	50	17.7
4. „ „ „ „ „ „ „ No. 1.	101	40	28.4
5. „ „ „ with narrow leaf blade and cut corolla No. 1	10	0	0*
6. „ „ „ „ „ „ „ „ „ „ No. 2.	10	0	0*
T. repens L.	18	0	0
T. incarnatum L.	20	15	75.0
T. resupinatum L.	15	14	93.4

* Cuttings were taken from plants in November, under snow.

Therefore the taking of cuttings in autumn, their preservation throughout winter and replanting in the following spring, without any weakening of parent form which has given the cuttings, enable us theoretically to prolong indefinitely the life of a clone and to use it in breeding for any successive number of years. This method, when applied to red clover, with some additional elaboration may be used also as a breeding factor, giving an estimation of the health of the cuttings and incidentally of that of the parent forms. The application of this method to the multiplication of variety, pure line or separate plants enables us to combine correctly the varietal testing of autogamous plants with their multiplication in a pure state.

(e) Outline of methods to be adopted in the breeding of red clover.

1. The obtaining of pure lines in isolators by means of insects which have been cleaned of pollen ; according to the actual conditions of work, the parent forms are either first multiplied vegetatively, crossed by hand, and only the first generation is taken into the isolator, or the parent forms are taken into the isolator without any preliminary work.

2. Pure lines obtained in the isolator are carried through varietal testing, parallel with which vegetative multiplication of pure lines is carried out by means of autumn cuttings. Material for cuttings may be taken from special sowings during the first years, when varietal testing is carried out in relatively small plots, and later from varietal testing fields the study of which has been completed.

3. Lines which have shown themselves to be the best are multiplied from the seed grown from cuttings in the largest possible plots, which guarantee the seed amounts necessary for large scale varietal testing, when the isolated plots are not smaller than 50 sq. m. It is better to scatter plots among sowings of plants other than clover, pollinated by the same insect species (*e.g.*, sainfoin, lucerne, lupin, etc.).

A Note on Nossovka Station.

The breeding of red clover at Nossovka was actually begun in 1925, when I. S. Travin was invited to take charge. The success of the work during the first years resulted in the recommendation by the Scientific Research Institute for Genetics and Plant Breeding (Odessa) and the First Conference of Plant Breeders in Odessa that the clover research should be extended, so that since 1929 a special clover section of the Plant Breeding Department has been in existence. The work with clover in Nossovka, however, has now terminated, and since the reorganisation of breeding work in U.S.S.R. it has been transferred to Belaya Tzerkov.

The following work done by Travin has been published by Nossovka Station. (In Bureau Library.)

(1) Breeding of red clover at the Nossovka Agricultural Experiment Station. No. 77. pp. 34. 1927. (Russian text.)

(2) Some new factors established in the course of work with red clover at the Nossovka Agricultural Experiment Station. No. 86. pp. 71. (English summary, 68-71). 1928.

(3) Breeding of Ukrainian red clover. (Ukrainian text.) 1929.

(4) Red clover breeding. (Report for 1925-1929.) No. 113. pp. 158. (Ukrainian text, English summary, 151-8.) 1930. (*Herb. Abst.* 1. 101. 1931.)

Nossovka Station is situated on 50° 56′ N. latitude and 31° 40′ longitude east of Greenwich, about 9 klm. from railway station. Elevation=136 m. Soil is leached black soil, northern variety, with 3.5 per cent. humus. Normal average rainfall in centimetres during fifteen years : Mar.=22.8 ; Apr.=38.9 ; May=46.8 ; June=60.1 ; July=82.0 ; Aug.=54.4 ; Sept.=49.1 ; Oct.=29.2.

R.O.W.

VI.—BRIEF NOTES ON SEED SETTING IN LUCERNE.

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Seed setting in lucerne is of vital importance to the plant breeder working with this crop, and a detailed knowledge of the factors affecting tripping and seed setting in the flowers is essential. Many factors, such as rainfall, soil moisture, temperature, humidity, sunshine, wind, insects, etc., have to be considered when determining methods for obtaining selfed seed.

The various methods of caging, bagging, etc., used to ensure self-fertilization of the flowers have a marked effect upon the degree of seed setting owing to the fact that the tripping mechanism is very sensitive to even slight variations in temperature and humidity. In an experiment in the season 1930-31, eight different types of cages were used for excluding bees, *viz.*, cheese cloth (open mesh), wire gauze, mill silk, mill silk and glass top, wire gauze and glass top, cheese cloth and glass, and unbleached calico.

From a temperature point of view, it is significant that the average temperature of the warmest cage (silk with glass top) was 2°F. below the normal sun temperature, when both the average minimum and maximum temperatures were compared. None of the cages gave temperatures as low as those recorded in standard louvred boxes.

The average maximum temperatures in the various cages used varied from 77 to 81.6°F., the lower average given being 5.6°F. lower than the average maximum sun temperature over the same period. Daily variations in the temperature differences between the cages ranged to 15°F. on very sunny days ; this would undoubtedly have a pronounced influence on the seed setting under the different types of cages. In some cases, low temperatures combined with high humidity and shading increased diseases such as rust or black spot, and decreased the amount of flowering, in the case of calico preventing it entirely.

In the cages with the higher temperatures where the flowering was almost normal, tripping and seed setting were decidedly reduced, although a practically normal rate of seed setting was obtained by resort to artificial hand tripping. From experience in New South Wales, strong transparent glassine bags are to be preferred for selfing single plants, as the flowers can be artificially tripped by a gentle stroking pressure from the outside. The flowers should be bagged in the unopened bud stage to prevent the carrying over of viable pollen from outside sources.

The opinion is rather loosely held that open artificial tripping ensures self-fertilisation, but this is not necessarily the case. Disposal of pollen by artificial or automatic tripping (aided by the wind) has been demonstrated fairly conclusively here, and this throws some doubt on the widely-accepted view that tripping by means of insects brings about cross-fertilisation and that automatic tripping results in self-fertilisation. On one occasion at Bathurst, an examination of the standards of untripped flowers disclosed the fact that all had at least a few adhering pollen grains ; the possibility of natural crossing without the direct influence of insects has been definitely established. Bees and other insects also play their part in this dissemination of pollen to the standards of untripped flowers. The length of time that this pollen remains viable on the standard must not be overlooked in the breeding technique employed.

A preliminary determination of the effect of temperature on the tripping of lucerne flowers was made. By the application of heat, produced by a magnifying glass or a lighted cigarette, automatic tripping is readily demonstrated. The reaction should therefore result from the loss of moisture and consequent diminution of cell tension. Dipping in 70 per cent. alcohol, presumably inducing similar cellular changes, caused almost immediate tripping.

Laboratory tests involving temperatures under varying conditions of humidity were also carried out. In an ordinary drying oven, the first tripping commenced at 101°F. and instantaneous tripping of all flowers occurred at 104°F. and upwards. Flowers suspended in steam over heated water commenced tripping at 102°F. Flowers actually immersed in heated water commenced tripping at 100°F. and with increasing facility as the temperature of the water was increased, until all the matured flowers tripped at a maximum temperature of 108°F.

These preliminary observations indicate that temperature exercises a very decided influence on the tripping of lucerne flowers, and that it must be a very important factor in determining what districts will be suited for lucerne seed production. They also demonstrate the importance of the lessening of temperature due to caging. It is interesting to note that the tripping commenced at approximately the same temperature under the most diverse moisture conditions.

The structure and physiology of the plant must receive close study both in the laboratory and the field. In the work at Bathurst, it has been found that sowing the selfed seed in proper seed beds with subsequent transplanting into properly spaced rows is the most satisfactory method for studying the progeny of selfed plants. Marked variations in the behaviour of selfed progenies to infection with downy mildew (*Peronospora trifolii*) and black spot (*Pseudopeziza medicaginis*) have been observed, some lines being much more resistant than others.

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Printed by the
Cambrian News (Aberystwyth), Ltd.